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Multidrug resistant tuberculosis – Diagnostic challenges and its conquering by nanotechnology approach – An overview

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ABSTRACT

One of the leading killer diseases that target the parenchymal tissues of lungs is Tuberculosis. Although anti-mycobacterial drugs are available, there are increased incidences of drug resistance encountered in *Mycobacterium* sp. They have been categorized into MDR (Multidrug resistant) and XDR (Extensively drug-resistant) strains exhibiting resistance toward successive treatment regimen. This situation threatens the futuristic containment of TB with the dearth of anti-TB drugs. Nanotechnology, the emerging multidisciplinary science has presented an excellent opportunity for timely and accurate diagnosis and discrimination of Mycobacteria via its unique physio-chemical and optical characteristics. The delayed and misdiagnosis of TB and lack of sensitive diagnostic method(s) has seen a paradigm shift toward nanoparticulate system for improved diagnosis, drug delivery and reduced treatment frequency. This review article highlights the evolution of tuberculosis and its transformation to multidrug resistant strain. Further, the conventional methods for diagnosing TB and the challenges encountered in their analytical performance have been highlighted and the strategies to overcome those challenges have been briefly discussed. Smart approaches encompassing metal nanoparticles, Quantum Dots (QDs) and Field Effect Transistors (FET) based biosensor for accurate diagnosis have been critically reviewed. A decade long state-of-the-art knowledge on TB nanodiagnostics, fabrication concepts and performance characteristics has been reviewed.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by bacterium *Mycobacterium tuberculosis* (*M. tuberculosis*/MTB). It most often target lungs that had spread through the air when TB patients cough, sneeze or spit. These airborne particles are termed infectious droplet nuclei which are found in the size range of 1–5 μm in diameter. Tuberculosis is caused not only by *M. tuberculosis* but members of MTB complex. They include *M. tuberculosis, M. bovis, M. africanum, M. caprae, M. microti, M. pinnipedii* and *M. canetti* [1]. Although preventable and curable disease, it was estimated that around 10 million people in 2018 were victimized due to tuberculosis.

According to WHO, nearly 1.5 million people die from this infectious disease every year which makes it the most infectious killer disease. Another data projects that only 5–15% of those who fell ill would be massively affected by TB disease (active cases). According to Global tuberculosis report 2019, there were over 30 countries with high TB burden (87%) among which eight countries constitute two-thirds of the total with India heading toward the top followed by China and Indonesia. Yet another transformation of TB is multidrug resistant (MDR) TB that poses a great threat worldwide with an estimate of 484 million new cases constituting 78% of rifampicin-resistant TB [2].

1.1. Multidrug resistant TB (MDR-TB)

Although TB is curable, there are several factors that have contributed at large for huge transformation into a drug-resistant strain. They include: poor quality drugs, poor hygiene, inappropriate use of drugs and premature-treatment approaches. In this line, MDR-TB could have emerged when the bacterium do not respond to the first line of drugs such as isoniazid and rifampicin. However, they may be treated using the second-line of drugs but which requires involvement of chemotherapeutic agents which are expensive and toxic. There is another serious level of drug-resistance i.e. Extensively drug-resistant TB (XDR-TB), a more serious form of MDR-TB that do not respond even to the second-line of drugs leaving the patients with no further treatment options (Fig. 1).

The drugs to which they develop resistance include ethambutol,

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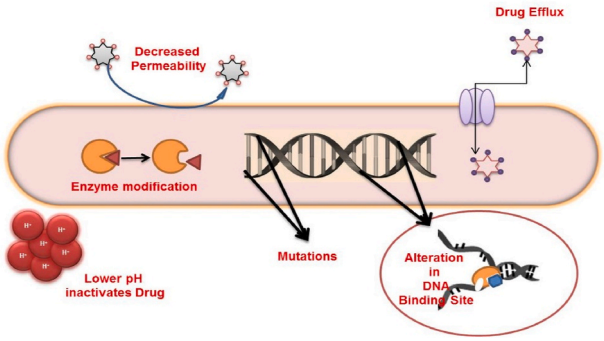
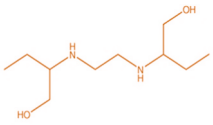
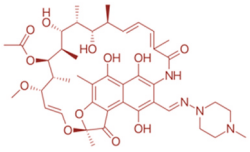
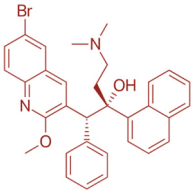
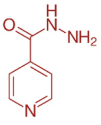
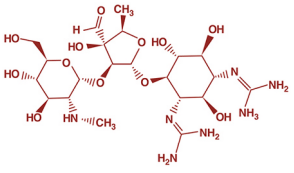
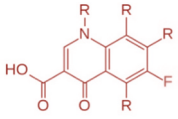
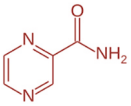
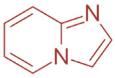


Fig. 1. Mechanism of Drug resistance in *M. tuberculosis*. (a) Decreased permeability – The inability of the drug to penetrate the cell wall; (b) Lower the pH renders the drug inactive from exhibiting its antibacterial effect; (c) Alteration in the enzyme pocket prevents the conversion of pro-drug to active form; (d) Mutations in DNA interfering with the DNA repair mechanism lead to the emergence of multidrug resistance; (e) Alteration in DNA binding site might prevent effective binding of RNA polymerase; (f) Efflux of the drug before reaching the target.

pyrazinamide, ofloxacin, ciprofloxacin, streptomycin, kanamycin and isoniazid [3,4]. According to WHO, it was estimated in 2018 that around 6.2% of MDR-TB cases had led to the emergence of XDR-TB and the remaining 56% of MDR-TB cases could be subjected to treatment. As a measure of global strategy and one of the targets for sustainable development, efforts in ending TB epidemic by 2030 have been initiated [2].

As the bacterium inhabits the host macrophage cells modulating the lysosome trafficking moiety, it hinders the formation of phagolysosome and thereby blocks the apoptotic pathway posing a great challenge in their containment. Secondly, there exist some limitations with the conventional drugs for its low bio-availability and bio-distribution which augment virulence (emergence of drug resistant strains) and mortality among humans. To surpass these pitfalls, either novel technological intervention or effective drug delivery strategies would be a promising approach in containing the emergence and dissemination of drug resistant strains of TB. This review highlights on the conventional molecular methods used for diagnosis and treatment of MDR-TB and the cutting edge technological intervention in overcoming the challenges posed by MDR strains for a more nano-based theranostics approach.

Table 1
Conventional Drugs used for the treatment of TB.

Pathogens	Drug/Antibiotics	Structure	Mechanism of action	Reference
M. tuberculosis/M. smegmatis	Ethambutol		Inhibition of cell wall synthesis (in particular, arabinan)	[5]
	Rifampin		Inhibition of RNA Polymerase activity	[6]
	Bedaquiline		Targets key respiratory chain enzyme F_1/F_0-ATPase and inactivates ion transporters.	[7]
M. tuberculosis	Isoniazid		Inhibition of cell wall/mycolic acid synthesis	[8]
	Streptomycin		A protein synthesis inhibitor	[9]
	Fluoroquinolones		Inhibits activity of topoisomerase IV and topoisomerase II preventing the detachment of gyrase from DNA.	[10]
	Pyrazinamide		A prodrug involved in the inhibition of membrane transport function in the bacterium.	[11]
	Imidazopyridine		Targets respiratory cytochrome *bc*1 complex and inhibits growth.	[12]

1.2. Origin of drug resistance

Drug resistance remains to be one of the biological phenomena observed in *M. tuberculosis* and tend to prevail since the discovery of streptomycin. Their continued use in treating patients made them to excrete resistant strains of *M. tuberculosis* in their sputum. To counterbalance the situation, drugs such as thioacetazone and para-aminosalicylic acid were introduced as combination chemotherapy to prevent the development of resistance. This chemotherapy lasted for 18 months to bring about effective anti-TB activity. It was with the advent of rifampicin (the most powerful anti-TB drug) that paved way for short-course chemotherapy. As a global initiative to combat TB, DOTS (Directly Observed Treatment Short-course) was introduced as a standard treatment regimen. Some of the conventional drugs used in the treatment of tuberculosis have been shown in Table 1.

1.3. Evolution of tuberculosis

The evolutionary origins of tuberculosis have a long-standing and challenging history owing to its etiological and social complications. It was hypothesized that MTB had survived over 70,000 years infecting more than 2 billion people all over the world. It had its origin 150 million years ago and the predominant ones belonged to the genus *M. ulcerans* capable of causing infections under specific environmental conditions. But, the early variant of the current genus MTB had its origin around 20,000 years ago. There was pool of evidences revealing the skeletal deformities related to TB infections and were found in Egyptian papyri. On the other hand, such kind of evidences (TB instances) were documented in India and China as early as 3000 years ago but had been referred in Biblical books using the Hebrew terminology '*schachepheth*' for describing Tuberculosis.

Tuberculosis was well documented by the Ancient Greeks in their book entitled '*Book I, of the Epidemics*' as *Phthisis* signifying the common characteristics of lung lesions. During the Middle ages, a novel form of clinical tuberculosis was reported and described as *scrofula* which was found associated with cervical lymph nodes. The same disease was named 'King's evil' in England and France that demanded 'royal touch' as the treatment and continued for several years till the British monarch of Queen Anne. Then, the medical intervention to treat tuberculosis was practised by French surgeon Guy de Chauliac by removing the scrofulous gland. With the identification of pathological signs (consolidation, pleurisy and pulmonary cavitation) of tuberculosis by French physician Theophile Laennec, a basic understanding on the vulnerable sites of infection was well established. In the due course of time, 19th century had ignited the exact etiology of tuberculosis once the term 'tuberculosis' was coined by Johann Schonlein and after a long-ending scientific debate. A brief overview on the evolution of tuberculosis according to the chronology has been cited in Table 2.

2. Diagnosis of tuberculosis

The procedures involved in the diagnosis of tuberculosis may be classified into three types that constitute conventional, immunological and novel diagnostic methods (Fig. 2). Conventional methods involve standard culturing methods and identification of the pathogen by using microscopic techniques. It is a rapid test presumptively used to detect the acid-fast bacteria present in the patient's sputum or other specimens and the results are read in one or two days [45]. Secondly, growth based approach pertains to the culturing of bacteria in the selective medium (Lowenstein-Jensen medium/Agar based Middlebrook 7H10) for a significant length of time (4 weeks) [46].

This is followed by Immunological methods namely ERBA LISA and SEVA TB ELISA to detect the IgG antibodies to A60 antigen and 31 kDa glycoprotein antigen respectively [47]. One of the most common methods of immunodiagnostics include Tuberculin skin test (Mantoux test) in which 5 TU of purified protein derivative is injected under the

Table 2
Evolution of Tuberculosis and its treatment regimen.

Timeline	Major Events	Reference
150 million years ago	Hypothesized on the origin of *Mycobacterium ulcerans*	[13]
3 million years ago	Early progenitor of *M. tuberculosis* infecting hominids in East Africa	[14]
20,000–15,000 years ago	Appearance of the common ancestors of the modern *M. tuberculosis* strains.	[15]
4500 years ago	Egyptian mummies with skeletal deformities with characteristic Pott's lesions were illustrated in Egyptian art.	[16]
3300–2300 years ago	Description of TB documented from India and China. Ancient Hebrews coined the word *schachepheth* to describe TB. In ancient Greece, Hippocrates termed TB as Phtisis. Aristotle described the contagious nature of TB as 'King's evil' in pigs and oxes.	[17–19]
1800 years ago	Marcus Aurelius recommended 'rest cure' involving fresh air, milk and sea voyages for successful treatment.	[20,21]
1200 years ago	'Scrofula' – a new clinical form of TB affecting cervical lymph nodes and its healing after a royal touch was described.	[22]
1712–1825	The practice of King's touch continued.	[23]
1363	Guy de Chauliac, a French surgeon proposed surgical removal of the diseased gland without harming vessels and nerves.	[24]
1679	Francis Sylvius described tubercles and its pathological and anatomical description.	[25]
1720	Benjamin Marten published 'A New Theory of Consumption' conjecturing the infectious origin of TB.	[26]
Mid 1700s	Johann Lukas Schönlein coined the term 'Tuberculosis' that replaced the then existing terms 'consumption' and 'phthisis.'	[27]
1854	Hermann Brehmer introduced 'sanatorium cure' and described Tuberculosis as a curable disease.	[28]
1865	Jean-Antoine Villemin demonstrated the infectious nature of TB.	[29]
1867	Theodor Albrecht Edwin Klebs attempted to isolate the TB bacillus using egg white medium	[30]
1882	Robert Koch used methylene blue staining recommended by Paul Ehrlich and successfully isolated, identified and cultivated *M. tuberculosis* in animal serum. He also reproduced the disease by inoculating them into laboratory animals. In 1905, he was awarded the Nobel prize in Medicine.	[31]
1890	Tuberculin, a purified protein from the bacterium was developed by Koch but proved ineffective as an immunizing agent.	[32]
1908	Charles Mantoux used tuberculin and developed an intradermic test for the diagnosis of tuberculosis.	[33]
1900–1928	Research work on developing anti-tuberculosis vaccine was initiated in 1900 by Albert Calmette and Camille Guerin. In 1908, a virulent strain *M. bovis* was cultured and sub-cultured on bile-glycerine-potato medium. In 1913, vaccination trials on cattle were interrupted by World war I. In 1919, they developed an attenuated strain that failed to produce tuberculosis when injected into guinea pigs, rabbits, horses etc. This marked the birth of BCG (Bacille Bilie Calmette-Guerin). In 1921, the first human administration of BCG to a healthy infant by oral route was initiated by Weill-Halle and Turpin. Oral route of administration was reported in infants without serious complications (1924–1928).	[34–36]
1944	Discovery of the promising antibiotic streptomycin (an anti-TB antibiotic) by Schatz, Bugie and Waksman. Alongside Jörgen Lehmann designed a chemical compound para-	[37,38]

(*continued on next page*)

Table 2 (*continued*)

Timeline	Major Events	Reference
	aminosalicylic acid (PAS) capable of killing the tubercle bacilli.	
1946–1950	Randomized clinical trials of combined Streptomycin and PAS by British Medical Research Council (BMRC).	[39]
1951	Discovery of Isoniazid by experts at Hoffmann-La Roche and Squibb, US and Bayer Chemical, Germany.	[40]
1952	Triple therapy (Isoniazid-Streptomycin-PAS) to combat all forms of tuberculosis.	[41]
1961	Ethambutol was developed and was found effective against isoniazid- and streptomycin-resistant organisms.	[42]
1966	The first clinical use of Rifampin in tuberculosis chemotherapy and was administered in combination with isoniazid and ethambutol that led to short term cure rates.	[43]
1970–1980s	Pyrazinamide was administered at lower doses had led to shortening of the course and further reduced to 6 months when combined with isoniazid and rifampin.	[44]

top layer of the skin, particularly on the forearm. The result is read by measuring the size of the lump developed after 48–72 h [48]. Yet another method for determining or detecting the presence of *M. tuberculosis* is by quantifying the levels of Interferon-γ or Interleukin-12 [49].

In recent years, there are innumerous novel technologies that are developed to detect the presence of *M. tuberculosis*. Some of them have been enlisted in Table 3.

3. Challenges and the need for nanotechnology

Although, identification of *M. tuberculosis* dates back to centuries, challenges in diagnosing TB still persist despite the technological advancements. The first and foremost criterion is its slow growth which limits its on-site patient care approach. Secondly, patients with pulmonary tuberculosis do not exhibit symptoms early during the infection which also leads to delay in diagnosis. Moreover, the difficulty encountered during the detection of low count of bacteria present in the sputum using staining, microscopy and other conventional techniques is yet another challenge that needs to be addressed. Finally, the success of diagnosis relies on the selection of complex samples such as sputum and other invasive body fluids on a par with blood and urine samples used in routine diagnosis [59–61]. Most importantly, the unavailability of biomarker(s), poor understanding of the host-pathogen interactions, pathogenesis and host immune responses further restricted their timely detection [62]. Besides the advent of novel molecular diagnostic techniques, the detection efficiency and the cost associated with Xpert MTB

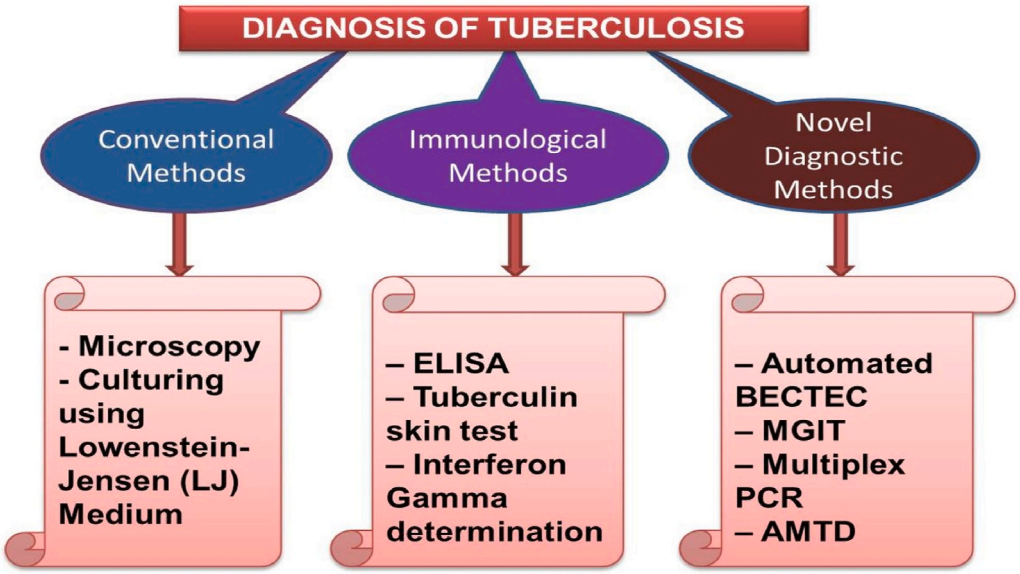


Fig. 2. Schematic representation of Tuberculosis Diagnostic methods: Conventional methods involve standard culturing techniques and identification by staining methods; Immunological methods involve either antigen or antibody detection using suitable bio-markers; Novel diagnostic methods constitute a more precise and accurate detection and differentiation of MTB.

Table 3
Molecular methods for the diagnosis of Tuberculosis.

Test	Type	Quality/Robustness	Reference
Fluoro type MTB	PCR based semi-automated direct detection of MTB within 3h.	Sensitivity – 88%; Specificity – 98%	[50]
GeneChip	RT-PCR based technique to detect Rifampin/Isoniazid drug resistance.	Sensitivity – 83–94.6%; Specificity – 91.3–98%	[51]
Genedrive MTB/RIF	Portable type RT-PCR to detect Rifampin resistance.	Sensitivity – 45.4%	[52]
GenoType MTBDRplus	Line probe assay for the detection of Rifampin and Isoniazid resistance.	Sensitivity – 90.3%; Specificity – 98.5%	[53]
LiPA MDRTB	Line probe assay for the detection of MTB resistant toward Rifampin and Isoniazid.	Sensitivity – 89%; Specificity – 99.4%	[54]
LiPA pyrazinamide	Line probe assay for the detection of MTB resistant to pyrazinamide.	Sensitivity – 65.9–100%; Specificity – 98.2–100%	[55]
MeltPro TB/INH	RT-PCR based closed tube (DeepMelt assay) technique for the detection of Isoniazid resistance.	Sensitivity – 95.7%	[56]
Xpert MTB/RIF ultra	Next-Gen cartridge based detection of Rifampin resistant MTB.	Sensitivity – 77–90%; Specificity – 96–98%	[57]
Truenat MTB	A chip-based NAAT (Nucleic acid amplification testing) with a portable RT-PCR.	Sensitivity – 99%	[58]

toward extra-pulmonary tuberculosis and HIV associated TB cases need careful consideration toward precision diagnostics employing simple protocols for rapid and durable detection [63]. With current TB diagnostics and their pitfalls (limited resources, lack of sophisticated instruments, technical expertise and the ultimate cost of diagnosis), there is an urgent need for the detection, the spread and treatment of MTB infection. In order to fulfil the unmet demands, nanotechnology based approaches would be a promising strategy as from the viewpoints discussed to offer rapid, reliable and efficient point-of-care (POC) TB diagnostics paving way for an effective treatment regimen.

It is a known fact that nanotechnology is revolutionizing all sectors of science and engineering employing nanoscale materials in the size range of 1–100 nm. As a general purpose technology, their applications are extended toward electronics, food & packaging industries and healthcare owing to their unique physio-chemical, optical, catalytic and antimicrobial characteristics. Most importantly, their application toward tissue engineering, drug delivery, bio-imaging and nano-diagnostics impregnating the concept of nanotechnology has bookmarked a massive growth in the biomedical sector. Alongside, their rapidity, sensitivity, specificity, robustness and affordability in the detection of proteins and metal ions have improved the performance characteristics of nano-diagnostic applications particularly in POC testing [64]. Evidence-based studies were also reported highlighting their disease detection efficiency. For instance, a bedside diagnosis approach using portable devices or mobile phones was used to detect HIV antibodies that aid Physicians to conclude rapidly on the diagnostic results [65,66]. In nano-based diagnostics, there are innumerous platforms that employ nanomaterials, quantum dots, dendrimers coupled to imaging, microfluidics, smart phones and microscopic systems to facilitate rapid and reliable diagnostic approaches for clinical applications (Table 4).

4. Nanodiagnostic methods for tuberculosis

The main objective of nanodiagnostics is to improve clinical diagnostic protocols with high sensitivity and accuracy by targeting the characteristic markers pertaining to timely detection. Secondly, to improve the reach of POC diagnostics in resource-poor and limited access areas in developing countries [70].

4.1. Nanoparticle based diagnosis

Nanoparticles fabricated using top-down and/or bottom-up approaches with size ranging from 1 to 100 nm have been used in combination with biological moieties. There are certain factors that make them compatible which include size of the particle, type of the metal precursor used for nanoparticle synthesis, its surface plasmon resonance (SPR), photoluminescence, electrical & thermal conductivity, stability and binding affinity. These aspects indeed govern the design and development of various nanobased devices/diagnostic kits [71]. Alongside, their ease of manipulation with respect to the type of biomolecules and organelles associated makes them an extremely potential drug delivery vehicle [72] (Fig. 3).

Table 4
Nanoplatform in the POC diagnosis of infectious agents/disease.

POCs based nano-diagnostic platforms	Components/Nature of Materials	Applications	Reference
Magnetic resonance based platform	MNPs and NMR system	A rapid and sensitive method to study host pathogen interaction or host responses.	[67]
Magnetic barcode based assay system	Magnetic nanoprobes and Microfluidic system/NMR.	Rapid, Sensitive and Specific. Detection of MTB and MDR TB within 2.5 h.	[68]
Paper strip based platform	Au/AgNPs impregnated in adsorbent pad and Lateral flow assay.	A portable, rapid, affordable and user-friendly platform with less sensitivity and accuracy. Multiplexed lateral flow assay capable of differentiating multiple pathogens.	[69]

4.1.1. Gold nanoparticles (AuNPs) based detection system

The unique characteristics of AuNPs such as biocompatibility, non-toxicity and inert nature have been the most sought after nanomaterial for biomedical implications. For instance, their optical property has been critically exploited to detect some of the clinically significant pathogens. Most importantly, the desired effect could be achieved without affecting the functional aspects of the system [73]. But it was Baptista et al. [74] who first utilized AuNPs and coupled them to DNA probes for the colorimetric detection of *M. tuberculosis*. The nanoprobes upon detection of complementary DNA turn pink and rather purple during their absence. This approach was found more accurate (InnoLiPA-Rif-TB) with 100% concordance ruling out the possibility of contamination, sample processing and rapidity (results could be read within 15 min). Furthermore, the same group has developed AuNPs derivatized thiol-modified oligonucleotides for the detection of single base mutations/single nucleotide polymorphism (SNPs) in *rpoB* locus to determine rifampicin resistant *M. tuberculosis* strains [75]. In addition, LAMP (Loop-Mediated Isothermal Amplification) was employed to detect MDR-TB with rifampicin resistance using Au-nanoprobes. This integration of LAMP and Au-nanoprobe assay facilitated early detection of rifampicin resistant MTB complex and the mutations associated with 100% specificity and sensitivity [76].

Au-Nanoprobes applications have been further extended to discriminate specific mutations associated with rifampicin and isoniazid resistance using a multiplex PCR reaction. Mutations associated with two loci containing *RpoB531* and *inhA(−)15* were targeted using specific Au-nanoprobe followed by a colorimetric assay coupled to a portable device for data analysis and screening [77].

Moreover, an upgraded version of paper based assay using AuNPs has been developed for the diagnosis of tuberculosis. This colorimetric sensing strategy employed unmodified AuNPs and single-stranded oligonucleotide probes for detection; data analysed by a sophisticated instrument; read out using cloud computing via smart phone. The LOD was found to be 2.6 nM of *Mycobacterium* target sequences with a turnaround time of 1h [78]. The same group have developed unmodified AuNPs-paper based colorimetric analysis utilizing the surface plasmon resonance (SPR) for MTB detection. Here, single stranded DNA probe gets hybridized to double stranded target TB DNA. The changes associated with hybridization would bring about variations in surface charge density of the colloid, indicated by a change in color. It is then readily measured to quantitate the DNA analyte. Prior to optimization, a LOD of 1.95×10^{-2} ng/mL of TB DNA could be detected with a turnaround time of 1 h [79].

Furthermore, Soo et al. [80] have developed a detection system with high flexibility, simplicity and efficiency at an affordable cost. They utilized AuNPs decorated thiol modified oligonucleotides for molecular diagnosis. The AuNPs probes GP1/GP2 and GP3/GP4 for IS6110 and Rv3618 respectively were developed to specifically target DNAs of MTB and MTB complex. This method had surpassed the conventional cultural and biochemical identification methods exhibiting 96.6% sensitivity and 98.9% specificity toward MTB complex detection and 94.7% sensitivity and 99.6% specificity in detecting MTB. Further, Hussain et al. [81] have developed a prototype using AuNPs for direct and rapid detection of MTB complex. In accordance, the 16S rDNA regions of MTB were amplified by PCR and the amplicons were targeted using oligo-targeters and AuNPs specific to genus and species level. Adopting this AuNPs-prototype, they could achieve detecting TB at 1 ng and 4 ng for

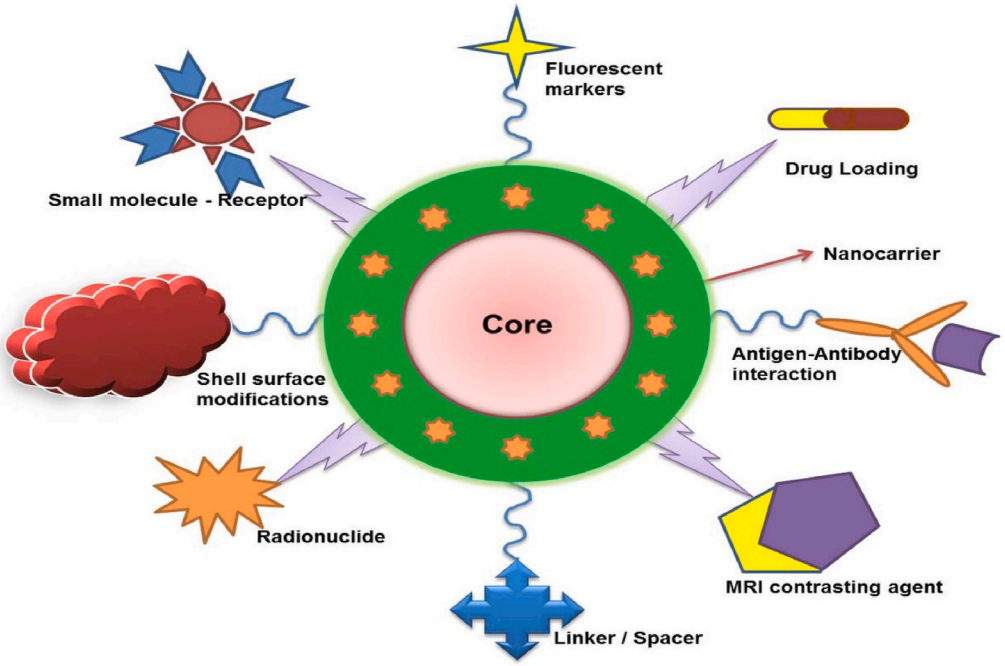


Fig. 3. Schematics of various applications and multifunctional modalities of Nanoparticulate system in human health viz. bio-imaging as contrasting agents, diagnostic/analytical tool, drug delivery and physical therapy.

PCR product and genomic DNA respectively within 1 h. This simple, rapid and inexpensive AuNPs based detection of MTB complex could substitute PCR-based detection.

More interestingly, Nucleic Acid Lateral Flow Immunoassay (NALF) superior to the conventional PCR has been developed for the rapid detection of MDR-TB. This approach uses AuNPs that are conjugated to anti-biotin antibodies for detecting the amplicons. Secondly, the anti-FITC/DIG antibodies are used to capture and immobilize the amplicons for visual detection. There are two test lines where one line codes for *RIF* resistance (*rpoB* assay) and/or *INH* resistance (*katG* assay) and other line for MTB control DNA [82].

4.1.2. Magnetic nanoparticle based detection system

Magnetic nanoparticles remain one of the naturally present nano-forms harbouring features for their remarkable use in nano-biomedicine and bio-imaging [83]. Their facile surface functionalization with recognition moieties viz. antibodies, antibiotics, polysaccharides etc. augment their application in diagnosis and detection of pathogens. In particular, SPIONs (Super paramagnetic iron oxide nanoparticles – $Fe_3O_4/\gamma\text{-}Fe_2O_3$) are used in great majority for drug delivery, cell tracking and bio-imaging by MRI [84,85]. Indeed, these iron oxide nanoparticles (IONs) have been conjugated with IgG for more efficient detection of bacteria in the limit of 10^4 CFU/mL using nano-MALDI. In this line, use of diagnostic magnetic resonance (DMR) along with IONs has been devised for the detection of MTB DNA [86].

A novel platform using magnetic beads has been devised for the detection of mutation at gene level. In this approach, streptavidin tagged magnetic nanobeads labelled with biotin have been developed for the detection of mutation in *rpoB* gene of MTB. An array of 11 padlock probes (PLPs) targeting 23S ITS region were involved in which two probes could detect MTB-complex and wild type respectively and the remaining nine probes to identify common mutations in RRDR-rpoB gene. This was designed based on the principle of Brownian relaxation and the signals were detected using AC susceptometry [87]. Further, the sensitivity and specificity of the MRI system have been improved by incorporating SPIONs. Molecular level host-pathogen interactions have been studied by developing a procedure involving the activation of SPIONs by anti-MTB surface antibody to form conjugates. This conjugated complex was once incubated with Mycobacterium followed by imaging. Specific target recognition would be revealed from the reduced signal intensity which could specifically detect extra-pulmonary TB i.e. central nervous system and abdominal TB [85,88].

Furthermore, advancement in harnessing magnetic nanoparticles has been evidenced in Magnetic barcode assays mimicking the functional aspects of Quantum dots (QDs). Here, specific complementary DNA sequences of MTB would serve as probes for more precise detection of TB [89,90]. This approach has simplified/nullified the need for DNA extraction and PCR amplification. Once the DNA is captured by the probes, the resultant conjugate would be fixed by the complementary MNP probes and were detected using NMR (Nuclear Magnetic Resonance) techniques [91].

4.2. Quantum dots (QDs) based TB diagnosis

Quantum dots are semiconductor particles of size less than 10 nm which have the fluorescence emitting properties under a wide range of wavelengths. Their fluorophore properties have been harnessed to detect and estimate the target cells/materials with great efficiency and accuracy. Alongside, their absorption, emission spectra and deliberate excited-state decay offers them more advantage over conventional fluorescence-based methods. Their ability to identify multiple targets has been greatly used in detecting clinical pathogens for use in disease diagnosis [92].

A fluorescent semiconductor based QDs and magnetic beads have been developed for the rapid detection of MTB. Two biotinylated oligonucleotide probes comprised of (a) Cadmium selenite QDs conjugated with streptavidin and a species-specific probe to emit fluorescent signal; (b) Magnetic beads conjugated to streptavidin and a genus-specific probe to separate and concentrate the targets were assembled. This method could detect the concentrated DNA target up to 12.5 ng/20 mL DNA. This method was applied to the detection of DNA extracted from *M. tuberculosis* and *M. avium* subsp. *paratuberculosis* isolated from Bronchoalveolar Lavage (BAL) and faecal samples. The accuracy of QD based assay when compared to real-time PCR was determined in the range of 70–90%. This enables easy, cost-effective and an accurate

method for the direct detection of MTB DNA in clinical samples [93].

Furthermore, the same group have designed an assay integrating Cadmium selenide QDs for the determination of surface antigens of MTB. The basic concept relied on the use of magnetic beads conjugated to genus-specific recombinant polyclonal and monoclonal antibodies for heparin-binding. This complex was then tagged with anti-mouse biotinylated antibody and streptavidin-conjugated QDs. Upon comparing the detection efficacy with *M. bovis, M. tuberculosis, E. coli* and *Salmonella sp*. (negative control) typical fluorescence was realized from *Mycobacterium* sp. with a minimum detection limit of 10^4 CFU/mL [94].

Similarly, immunomagnetic beads combined with functionalized QDs technology was proposed by Ma et al. [95]. Initially, nanobeads and QDs were prepared by using wet chemical method and conjugated to MTB binding peptide H8 for specific reaction toward $H_{37}R_v$. Upon reaction, a ternary complex was formed with $H_{37}R_v$ at 100 mg/L QDs with a turnaround time of 2h. The LOD of $H_{37}R_v$ in suspension and simulated sample was determined to be 10^3 CFU/mL that could discriminate them from non-mycobacteria. Yang et al. [96] have fabricated unique method of detecting MTB using surface functionalized magnetic nanospheres (MNSs) coupled to QDs specific to $H_{37}R_v$ peptides. The conformational changes associated with the bacteria-magnetic microspheres-QDs emit fluorescence while the separation is facilitated by the MNSs. The LOD was determined to be 10^3 CFU/mL capable of discriminating the test sample from control sputum samples.

In this line, Kim et al. [97] have developed an easy and a sensitive method based on antigen-antibody reaction incorporating silica-decorated QDs and Au nanorods. These nanomaterials were involved in the detection system without any surface modifications and to which genetically engineered antibodies viz. GBP50B14 and SiBP8B3 specific to Ag85B (MTB specific secretory antigen) were bound. Upon successful antigen-antibody reaction, the quenching effect in the presence of a target was determined. The assay response was determined to be 1×10^{-3} to 1×10^{-10} μg/mL. The LOD of the antigen was found to be 13 pg/mL.

Huang et al. [98] have developed a rapid and a sensitive CdS quantum dot (QDs) coupled to magnetic beads based immunosensor for the detection of INF-γ associated with tuberculosis. The fabrication has been done using carbon electrode completely covered by AuNPs upon which antihuman IFN-γ antibodies were immobilized. Quantification of cadmium yielded the amount of analyte captured using square wave anodic stripping voltammetry (SWASV). A linear relationship in the electrochemical signal was found in the range of 1 pg/mL to 500 pg/mL with a LOD of 0.34 pg/mL.

Zhou et al. [99] have developed an immunosensor for the detection of triple latent tuberculosis infection markers using carbon QDs and CdS QDs respectively. These QDs were integrated onto AuNPs to fabricate ECL nanoprobes for high sensitive detection of IFN-γ, TNF-α and IL2. These three markers were immobilized on three patterned areas of indium tin oxide electrode facilitating easy capture of markers. The electrochemical signals resolved upon successful capture could detect the concentration of the markers at the range of 1.6–200 pg/mL.

4.3. Biosensor based diagnosis

A biosensor is an instrument devised to qualitatively/quantitatively analyse the biological entities by coupling them with a bio-recognition elements such as amplifiers, transducers etc. The physico-chemical/electrochemical changes induced upon sensing attributes for the detection of the target [100]. A schematic on the working of a biosensor is shown in Fig. 4.

Initially, a piezoelectric based biosensor was established to detect *M. tuberculosis* directly from the clinical samples. This system adopted the use of quartz crystal immobilized on biotinylated gold surface. Upon addition of the sample, the presence of target if any gets hybridized to the DNA biotinylated probe in the system. The LOD of DNA was determined in the range of 0.5–30 μg/mL [101].

Similarly, a highly sensitive amperometric nucleic acid biosensor was developed by Das et al. [102]. Here, an oligonucleotide probe containing single stranded DNA was immobilized to ZrO_2 nanoparticles deposited on gold electrode specifically for the detection of *M. tuberculosis*. The electrocatalytic response in the event of detection of analyte was determined to be 0.065 ng/μL taking not less than a minute. The same group have designed an impedometric biosensor utilizing ZrO_2 nanomaterials deposited onto multi-walled carbon nanotubes (MWCNTs). This ultrasensitive method facilitated rapid electrochemical biosensing of target DNA [103]. A step further, He et al. [104] have synthesized silver nanoparticles in a single step using luminol and developed an electrochemiluminescence based sensor for the detection of *M. tuberculosis* DNA.

Apart from the genetic material, the volatile compounds released by a bacterium in a liquid medium could also be detected using electronic nose-based biosensor. There are three different chambers namely (a) volatile gas chamber (b) a sensor array and (c) a data analysis system. Here, metal oxide based semiconductors comprising metal-oxide semiconductor field-effect transistors (MOS-FETs), ZnO, MnO, TiO_2, SnO_2 and conducting polymers are integrated in a transducer based system. Once, any volatile compound is released, significantly different from that of the patterned sensor array is analysed from the signals. It could differentiate *M. tuberculosis* from *M. avium, M. scrofulaceum, Ps. aeruginosa* etc. A product, *Aeonose,* one of the commercial e-nose sensors is under development by a Dutch company [105].

The diagnostic potential of MTB specific antigen present in the

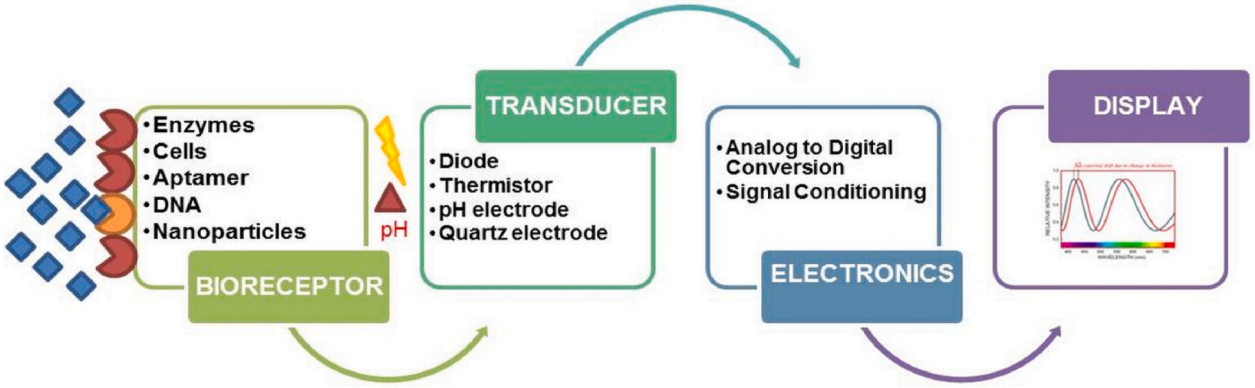


Fig. 4. Biosensor - mechanism-of-operation. Bio-sensing mechanism is based on the interaction of an analyte with the receptor probes. Upon successful interaction, the transducer measures the interaction and quantifies by a signal. The intensity of the signal detected is proportional to the concentration of the analyte. Then this signal would be amplified and processed by the electronic device for visualization.

culture filtrate protein, CFP10 and its early detection was validated using culture magnetophoretic immunoassay. This approach adopts MNPs decorated with AuNPs and coated with anti-CFP10 antibody with a LOD of 0.3 pM [106]. A similar approach has been developed to detect the latent TB markers IFN-γ, TNF-α and IL-2 using electrochemiluminescence-based immunosensor. This immunosensor employs luminol, carbon and CdS QDs which were integrated onto AuNPs and immobilized onto magnetic beads. These beads were separately conjugated to secondary antibodies specific for each marker. Whereas the primary antibodies were immobilized onto indium tin oxide electrode to capture corresponding analytes present in the serum. Upon successful binding of magnetic beads to the analyte, electrochemiluminescence signals would be generated based on the intensities corresponding to the concentration of the analytes. Using this biosensor, the markers as low as 1.6–200 pg/mL could be detected [107].

Besides gold and other semiconductor nanoparticles, magnetic nanoparticles (MNPs) have also been harnessed in the detection of MTB in a typical biosensor. MNPs functionalized with specific antibodies have profound implications in magnetoresistive biosensors for MTB detection. For instance, Streptavidin-coated MNPs with anti-MTB biotinylated antibodies were developed to capture BCG. Once the BCG is captured onto the surface of the biochip, a small change in the magnetic field triggers an array of spin-valve sensors attributing for an effective POC method for MTB detection [108].

5. Nanotherapeutics

Nanotherapeutics in combatting TB holds great promise in infectious disease management that would benefit human society. Advancement in technology has allowed novel formulations, drug delivery, diagnostics and therapy. There are many advantages in using nanobased systems in which a greater efficacy could be achieved administering lower dose of the drug with fewer side effects in a short turnaround time [109,110]. Alongside, targeting efficiency could be improved by enhancing the drug concentration at specific sites while leaving the surrounding environment undisturbed. With active targeting, the nanoparticles are made to conjugate with antibodies, specific ligands or surface modified so that they are prevented from binding to non-targeted sites and thus targeting only specific receptors [111]. For instance, PEGylation of the nanoparticle renders them hydrophilic which enhances the circulation time, preventing aggregation and uptake by the reticulo-endothelial system. Secondly liposomal encapsulation may prevent them from enzymatic degradation, improves stability and controlled release at the targeted site [112].

The fine tuning of the size of the nanoparticles on a par with the intracellular molecules and organelles enhances the interaction possibilities at cellular and sub-cellular level. More importantly, there is a ready uptake (of nanoform of the drug) by the macrophages through phagocytosis greatly helps in treating some of the infectious diseases. For instance, AgNPs exhibit antibacterial action through (a) direct contact (b) release of Ag + ions, (c) interrupting metabolic processes/pathways (d) ROS generation (e) cytotoxicity, (f) genotoxicity and (g) membrane disruption [113,114].

There is an emerging practise of combinatorial therapy to encounter tuberculosis. In this approach, the potential nanoparticle (TiO_2, CuO, Au, Ag, ZnO) would be combined with an antibiotic (Nanoantibiotics) for an enhanced antimycobacterial effect to counterbalance the situation of emerging antibiotic resistance [115].

Liposomes are yet another approach that facilitates easy delivery of anti-TB drugs. Liposomes offer '*enhanced permeability and retention effect*' for their high level of selectivity. They have been used as a carrier for common anti-TB drugs such as gentamicin, sparfloxacin, amikacin, streptomycin and others (Table 5).

Table 5
Liposome based Anti-TB drug delivery system.

Drug	Liposome Type	Antimycobacterial effect	Advantages
Gentamicin/ Sparfloxacin	Lipid	*M. avium* complex	Studies were conducted in Beige mouse where reduced viability in spleen and liver cells were observed.
Amikacin	Ethanol injection	*M. avium* complex	Studies conducted in Murine mouse showed reduced bacterial replication and controlled release.
Streptomycin/ Isoniazid/ Rifampicin	Multilamellar liposomes	*M. avium*/ *M. tuberculosis*	Studies conducted in Beige mouse showed increased chemotherapeutic effect.
Pyrazinamide	Dipalmitoyl PC	*M. tuberculosis*	There was a significant therapeutic efficacy when tested using mouse model.
Clofazimine	DMPC/DMPG	*M. tuberculosis*	Studies conducted using BALB/c mouse showed biocompatibility with reduced CFU.

PC – Phosphatidylcholine; DMPC – L-α-dimyristoylphosphatidyl choline; DMPG - L-α-dimyristoylphosphatidyl glycerol.

5.1. Therapeutic strategies

Nanotechnology has been one of the most promising strategies challenging against multidrug resistant strains of bacteria (that includes MDR- and XDR-TB) with their size, shape and concentration. The greatest achievement relied on reaching the target site with their size when conventional antibiotics failed to do so. Reports on enhanced antibacterial effect against multidrug resistant strains by metallic nanoparticles highlighted its broad spectrum potential [116]. Moreover, the multifunctional magnetic nanoparticles (IgG-Fe_3O_4–TiO_2) showed remarkable antibacterial activity against pathogenic bacteria under the influence of UV irradiation by disrupting the front-line barrier causing cell death.

There are other metal oxide nanoparticles namely PVA-coated ZnO NPs that induce oxidative stress by their internalization and some exhibiting their maximum antibacterial effect in a dose-dependent fashion [117]. Copper nanoforms were reported to efficiently challenge clinical strains in hospital and community settings [118,119]. It was reported that NPs-mediated dissipation of cell membrane potential to be the probable reason for the antibacterial effect in addition to generation of reactive oxygen species, lipid peroxidation, protein oxidation and DNA degradation. The *modus operandi* involved in the metal nanoparticle-microbe interaction has clearly been elucidated in Fig. 5.

Nanoparticle synthesis plays an important role in classifying them into organic and inorganic based on the route of synthesis. Besides physical and chemical methods, biological route of synthesis is gaining much importance by adopting enzymes, metabolites etc. adhering to green chemistry principles and in eco-friendly manner. This route suffers from size controllability and downstream processing [120]. More importantly, these metallic nanoparticles are used as carrier with significantly higher loading efficiency and potential antibacterial effect. In this line, chitosan-AgNPs exhibited excellent antibacterial effect against MRSA within a short period accompanied by a significant decrease in colony forming units when compared to MSSA. Similar results were reported for multidrug resistant strains of *K. pneumoniae* and antibiotic susceptible *E. aerogenes* strains [121].

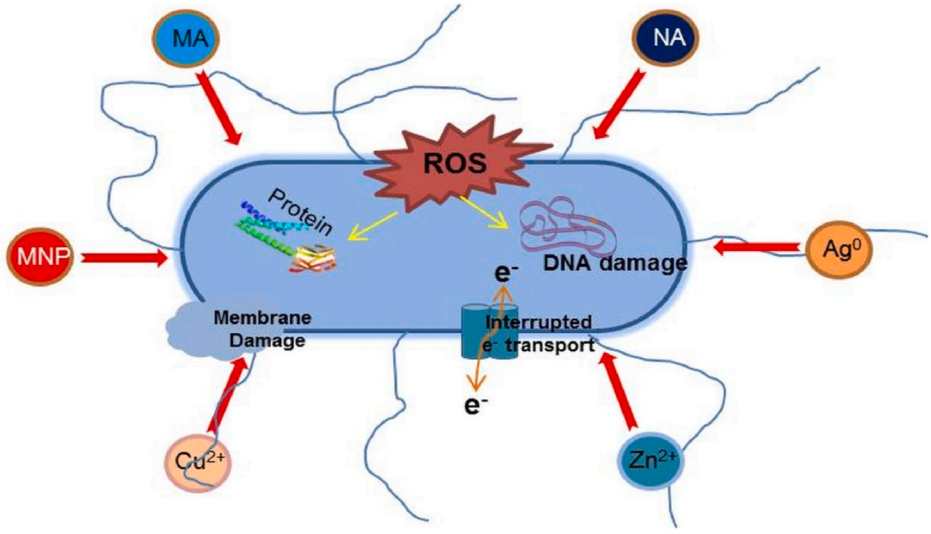


Fig. 5. Possible antibacterial mechanisms of nanoparticle interaction with the bacterial pathogens. By electrostatic interaction, the nanoparticle comes in contact with the bacterial membrane and by the release of its ions damages the membrane leading to leakage of cell contents. Upon entering the cell, they have a broad target range where they interact with the protein and DNA causing oxidative damage and thereby disrupting transcription and translation. They interrupt the respiratory chain reaction by interacting with the sulfhydryl groups inducing lipid peroxidation. (MNP – Multifunctional nanoparticle; MA – Metal nanoparticle – antibiotics conjugate; NA – Nanoantibiotics).

Further, the synergistic effect of metallic nanoparticles-antibiotics against multidrug resistant strains was manifold when compared with only antibiotic treatment to improve the antimicrobial efficacy [122]. It is noteworthy to mention that in hospital settings, the metallic nanoparticles are incorporated in the development of medical devices, dental implants, catheters, prostheses and bone cement as long-acting biocidal agents [123].

Polymeric nanoparticles had been the recent interest in effectively delivering the antibiotics with utmost stability. Polylactide-co-glycolide (PLGA) exhibited excellent carrier efficiency and employed in nanotechnological drug delivery applications [124]. Synthesis of PLGA mediated nano-bacitracin in the form of core-shell had showed an increased antibacterial effect as with free bacitracin [125]. Similarly nanoantibiotics prepared by incorporating imipenem in PLGA or PCL nanocapsules showed enhanced antibacterial and anti-adherent properties in the evaluation of imipenem-resistant strains [126].

In this sequel, liposomes, solid lipid nanoparticles, nanoceramics have received attention as site-targeted nanotherapeutics. Bacteriophages, with their unique specificity and bactericidal potential had been a promising approach to tackle antibiotic resistant strains [127]. During oral administration, encapsulation of the phages with nanocarriers would help escape the digestive and immune systems or binding them to macroscopic structures confers insolubility; these combinational approaches had been potentially used in the eradication of antibiotic resistant strains [128,129]. The antimicrobial strategies of nanomaterials as stand-alone and in combination with polymers and antibiotics have been included but not limited to encountering Enterobacteriaceae members and may be applied to contain resistant strains of bacteria (MDR TB/XDR TB) in near future. Furthermore, no such ground-breaking studies have been proved in humans and studies pertaining to treatment regimen are still under infancy that demands in depth investigation.

6. Conclusions and future outlook

In the fast moving world, there are several slow growing infectious agents/diseases that have the potential to victimize human race on a long run. One among them is tuberculosis which still poses a great challenge to health care sector in detecting/diagnosing them. This may be due to the unavailability of proper detecting system, poor awareness among public and poor detection sensitivity of the bacilli in clinical samples. Nanotechnology, an interdisciplinary field which emerged in recent years has paved ways and helped resolve many hurdles and healthcare challenges in diagnosis and treatment of infectious diseases. With the ease of manipulation, miniaturization of devices their application had helped manage TB offering rapid diagnosis at an affordable cost with limited resources. In this review, an overview of the recent trends in the nanotechnology enabled diagnosis of TB and MDRTB has been described. The diagnostic perspectives with regard to nanoparticles, fluorescence based Quantum dots and nanobiosensors surpassing the pitfalls over conventional methods have been critically reviewed.

There is no doubt that nanotechnology has given some valuable solutions to unresolved medical challenges. The criticality relies on timely, accurate detection of TB through potential biomarkers and to differentiate them from other strains. Alongside, a convenient, affordable nanobiosensing platform and patient friendly POC for detecting mycobacteria need to be developed which would be a major breakthrough in TB theranostics.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbi.2021.109397.

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